

P-25 Effects of Epidermal Growth Factor, Transforming Growth Factor Beta and Gonadotropin on In Vitro Maturation of Porcine Oocytes

**Sang Jun Uhm, Jin Young Joo, Ji Yun Chang, Nam-Hyung Kim,
Hoon Taek Lee and Kil Saeng Chung**

Animal Resources Research Center, Kon Kuk University, Seoul 143-701, Korea

Epidermal growth factor (EGF) and transforming growth factor beta (TGF β) have been considered as potential regulators of meiotic and cytoplasmic maturation of porcine oocytes. However, little information is available on their intergrated roles and interactions with gonadotropins and other components of follicular fluid during meiotic maturation. The objective of this study is to determine the interactive effects of EGF, TGF β and gonadotropin on the meiotic maturation in the presence and absence of porcine follicular fluid (pFF). Radioimmunoassay revealed that the pFF used in this study contained with 6.1 ng/ml estradiol-17 β , 15.61 ng/ml testosterone and 35.7 ng/ml progesterone. Cumulus-oocyte complexes from slaughterhouse-obtained porcine ovaries were matured in TCM 199 medium containing 10 ng/ml EGF, 1 ng/ml TGF β and/or 10 mg/ml FSH for 44 h at 39 $^{\circ}$ C under an atmosphere of 5% CO $_2$ and 95% air with high humidity. In the absence of pFF, EGF alone or incombination with FSH stimulated oocytes maturation, while TGF β decreased incidence of meiotic maturation. In the presence of pFF, in contrast, the inhibitory effect of TGF β on the meiotic maturation was reduced. However the stimulatory effects of EGF alone was not changed in the presence of pFF. These results suggested that the stimulatory and inhibitory effects of EGF and TGF β on the oocytes maturation were intergrated with gonadotropin and other components of follicle in the pig.

P-26 Comparison of microtubule organization and chromatin configuration in bovine oocytes following intracytoplasmic injection with spermatozoa and isolated sperm heads

**Soo Hyun Jun, Jung Tae Do, Jong Tae Choi, Mi Yong Chung,
Nam-Hyung Kim, Hoon Taek Lee and Kil Saeng Chung**

Animal Resource Research Center, Kon Kuk University, Seoul 143-701, Korea

Microtubule assembly and chromatin configuration were compared in bovine oocytes following intracytoplasmic spermatoozoon and isolated sperm head injection. Microtubule localization was confirmed using a mouse monoclonal antibody to α -tubulin and detected using as fluorescent labeled goat anti-mouse secondary antibody. DNA was stained with propidium iodide. The image of microtubules and chromatin was captured using laser scanning confocal microscope. Soon after a spermatozoon injection, microtubular aster was seen adjacent to the sperm neck area. The sperm aster enlarged and, at the time of pronuclear apposition, filled the cytoplasm. In contrast, microtubule aster was not seen in the all case following sperm head injection. Instead, a dense

network of microtubules in the cytoplasm was organized from the cortex in normally fertilized eggs. During pronuclear movement, the maternally derived microtubules filled the whole cytoplasm, which appeared to move male and female chromatin. Chromosome analysis revealed that some embryos, which were derived from sperm head injection, contained diploid chromosomes. These results suggested that isolated spermatid nuclei of the cattle can develop into a morphologically normal pronucleus in matured bovine oocytes and are competent to participate in syngamy with the ootid chromatin. Functional microtubules for the complete fertilization with isolated sperm head were not associated with male derived centrosome, but organized solely from maternal components.

P-27 생쥐 1세포기 수정란의 동결보존 후 배발달에 관한 연구

가야병원 불임 클리닉

김재명 · 고영호 · 조해영

난자의 동결보존 과정에 있어 난자는 일련의 생화학, 물리적 장애를 입는 것으로 알려져 왔다 (Ashwood-Smith et al., 1988). 동결이 실시되는 동안 난자의 세포막 내 또는 외에 이들의 영향을 받는다. 따라서 동결보존에 있어 난자의 발달단계, 동결 보호제의 종류, 동결방법에 따라 동결보존 후 난자의 생존 및 발달에 중요하게 영향을 끼치는 것으로 알려졌다. 여러 보고에 따르면 PROH가 인간 및 생쥐 초기배에 높은 성적을 나타 낸다고 하며 (Renard & Babinet. 84 Lassalle et al., 1985; Mandelbaum et al., 1987), 2,4,8 세포기와 같은 지수 단계의 난자가 3,5,6,7 세포기 같은 분열단계의 난자보다 동결 및 융해시 더 높은 생존성과 배발달율을 나타낸다고 하였는데 즉 이것은 핵상이 휴지기인 난자가, 세포내 핵물질이 산재되어 있는 난자보다 동결보존과 같은 외부의 영향에 충격을 덜 받는다고 하였다 (Lassalle et al., 1985; Testart et al., 1986, 1987).

Smith (1952)는 이같은 현상이 Syngamy와 Segmentation이 일어나는 시기에도 발생 한다고 했으나, Mandelbaum 등 (1987)과 Hartshorne 등 (1990)은 확인할 수 없었다.

수정란이 분열함에 따라 Cell cycle에 있어서 핵 분열은 매우 짧은 기간에 발생하며, G₂ 기간이 길어진다. 난자의 동결보존에 있어 방추사와 염색체의 손상을 피하기 위해 간기의 난자동결을 권유하며 (Ng et al., 1988; Chedid et al., 1992), Cell cycle이 S기와 G₂기인 4세포기 생쥐난자를 1,2-PROH로 동결시 G₂기 에서 높은 성적을 얻었다 (Balakier et al., 1991).

정자가 난자내로 침투하여 난자가 활성화 되면 (Longo, 1980), 제2극체를 방출하고, 피질과립 (Cortical granule)의 exocytosis가 일어나 투명대가 경화되어 정자의 다정자 침입을 억제한다 (Lopata et al., 1980; Wassarman, 1987). 웅성전핵은 난자에 정자가 침투된 부위 주변에서 형성되는 반면, 자성전핵은 감수분열 중기의 방추사 난자내 극성(ooplasmic pole)에서 형성 된다(Longo, 1973, 1987). 형성된 두핵은 난자의 중앙을 향하여 두 핵사이가 밀접하게 연합되며, DNA복제가 이루어 진다(Longo, 1976; Schatten et al., 1985). 즉 이와같이 전핵기 난자에서 이런 일련의 현상이 발생하는데, 수정란에 있어서 전핵의 유무 및 hCG주사 후 시간에 따라 동결보존후 융해하여 1세포 수정란의 생존성 및 배발달율을 조사 하였다.